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Serum β_2-microglobulin levels in asymptomatic HIV-1-infected subjects during long-term zidovudine treatment

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Abstract

β_2-microglobulin levels were determined in the serum of 18 initially asymptomatic HIV-1 p24 antigenaemic subjects who were treated with zidovudine (± acyclovir) and who were followed for 2½ years. The median serum β_2-microglobulin level at week 0 was 2·5 mg/l and decreased to 2·3 mg/l after 12 weeks of treatment ($p = 0·001$). A correlation was found between individual changes in serum β_2-microglobulin levels and individual changes in serum p24 antigen levels during the first 48 weeks of treatment ($p < 0·05$). Six out of 18 subjects progressed to AIDS after 60–126 weeks of treatment. In this group during a period of more than one year before disease progression median serum β_2-microglobulin levels increased from 2·5 mg/l to 3·3 mg/l ($p = 0·03$) and median CD4+ cell counts decreased from $0·3 \times 10^9$/l to $0·08 \times 10^9$/l ($p = 0·03$), while in that period the pattern of serum p24 antigen levels was inconsistent. Although the variability in serum β_2-microglobulin levels appeared to make this marker unsuitable for management decisions in individuals, a decline in β_2-microglobulin levels was found to parallel a decline in p24 antigen levels during the early phase of zidovudine treatment. Moreover, after prolonged treatment, rising β_2-microglobulin levels—in contrast to p24 antigen levels—were shown to have predictive value for disease progression.

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Introduction

Treatment with the thymidine analogue zidovudine (3'-azido-3'-deoxythymidine, AZT) has been found to be of clinical benefit for patients with the acquired immunodeficiency syndrome (AIDS) or AIDS-related complex (ARC), and for asymptomatic subjects with human immunodeficiency virus type 1 (HIV-1) infection with less than $0·5 \times 10^9$/l circulating CD4+ cells.[1–3] A temporary increase in CD4+ counts was seen in groups of zidovudine-treated subjects as opposed to a sustained decrease in groups of untreated subjects.[1–3] Serum levels of HIV-1 p24 antigen in zidovudine-treated subjects showed a decline in contrast with those of untreated subjects.[1–5] P24 antigen is a specific marker for the monitoring of antiretroviral therapy, but it is not very sensitive: only 40–60% of patients with AIDS, and 12–25% of asymptomatic HIV-1-infected subjects have detectable circulating p24 antigen.[4 6–8] At present the most sensitive and specific method for quantifying the in vivo antiretroviral activity appears to be the measuring of HIV-1 titres in plasma by end-point-dilution cultures; however, this method is too laborious for routine use.[9]

β_2-microglobulin in a low molecular-weight polypeptide chain, present as a part of the class I major histocompatibility complex on the surface of most somatic cells.[10] β_2-microglobulin has been reported to be elevated in viral infections (including HIV-1 infection) and haematological malignancies.[6 8 11 12] In HIV-1-infected subjects serum β_2-microglobulin levels are strongly correlated with the risk of disease progression to AIDS, probably by immune activation and turnover of CD4+ lymphocytes and macrophages resulting from HIV-1 infection.[6 8 11 12] In a group of asymptomatic HIV-1-infected homosexual men the relative hazard for progression to AIDS within 3 years for subjects with serum β_2-microglobulin levels from 3·1–5·0 mg/l was 4·5 compared with subjects with levels $\leq$ 3·0 mg/l.[6] Jacobson *et al* suggest that the serum β_2-microglobulin concentration, which is simple to measure, is a marker that could be used for the monitoring of an antiretroviral effect in all HIV-1-

infected subjects.[13] They showed a statistically significant decrease in serum β_2-microglobulin concentrations in AIDS and ARC patients treated with zidovudine for 24 weeks.[13] Individual changes in serum β_2-microglobulin concentration correlated with individual changes in serum p24 antigen level.[13]

Serum β_2-microglobulin could be a particularly useful marker for studies with antiretroviral drugs in asymptomatic HIV-1-infected subjects, because the great majority of those subjects do not have detectable serum p24 antigen.[4 6-8 13] We therefore analysed serum β_2-microglobulin levels in 18 HIV-1 p24 antigenaemic subjects in whom zidovudine treatment was started in the asymptomatic phase and who were followed for 2½ years; six of them developed AIDS during the follow-up period.

Subjects and methods

Subjects Eighteen persistently HIV-1 p24 antigenaemic subjects, who were either without symptoms (CDC group II)[14] or were suffering from persistent generalised lymphadenopathy (CDC group III)[14] have been treated with zidovudine in an open label study, as previously described.[15 16] Treatment schedules were as follows: group A (n = 6) was treated with zidovudine 1000 mg daily, group B (n = 6) was treated with zidovudine 1000 mg and acyclovir 3600 mg daily and group C (n = 6) was treated with acyclovir 3600 mg daily for 8 weeks, then with zidovudine 2000 mg daily for 4 weeks and subsequently with zidovudine 1000 mg daily. Twelve subjects remained asymptomatic during 130 weeks of treatment. Two of them, one from group A and one from group B, developed symptomatic anaemia and had dose reductions of zidovudine and short dose interruptions, as previously described.[16] Six subjects showed disease progression to AIDS, four of them developed *Pneumocystis carinii* pneumonia at weeks 60 (from group A), 80 (group B), 90 (group C) and 93 (group B) and two of them oesophageal candidiasis at weeks 112 (group C) and 125 (group C). No primary prophylaxis against *Pneumocystis carinii* was given during the study period. The subject with disease progression at week 60 had, before AIDS was diagnosed, dose reductions of zidovudine and short dose interruptions because of anaemia, as previously described.[16]

During treatment subjects were seen at least 4-weekly for clinical and laboratory evaluation. Serum samples for p24 antigen and CD4+ lymphocyte counts were obtained at least at 12 weeks intervals. The β_2-microglobulin concentration was measured in samples of frozen serial serum specimens taken at weeks 0, 12, 24, 48, 72, 96 and 120 (+/− 2 weeks). In the subjects with disease progression to AIDS the last serum β_2-microglobulin sample measured before the moment of diagnosis was chosen as last evaluation point.

Table 1 Serum β_2-microglobulin levels, serum p24 antigen levels and CD4+ cell counts in all subjects (n = 18, week 0–48), the non-progressors (n = 12, week 0–120) and the progressors (n = 6, week 0–48); medians, ranges and p values compared with pretreatment values (Wilcoxon signed rank test)

Week	*0*	*12*	*24*	*48*	*72*	*96*	*120*
β_2-microglobulin (mg/l)							
All subjects	2·5	2·3	2·4	2·4			
	1·4–4·1	1·3–3·2	1·2–3·4	1·5–3·8			
	—	0·001	NS	NS			
Non-progressors	2·3	2·1	2·3	2·5	2·3	2·4	2·6
	1·4–3·8	1·3–3·2	1·3–3·4	1·5–3·8	1·7–3·7	1·5–4·9	1·8–4·9
	—	0·01	NS	NS	NS	0·01	NS
Progressors	2·8	2·4	2·5	2·4			
	2·3–4·1	1·7–3·0	1·8–3·1	1·7–3·5			
	—	0·05	NS	NS			
P24 antigen (pg/ml)							
All subjects	286	108	65	83			
	66–3746	17–817	15–460	14–750			
	—	0·002	0·002	0·002			
Non-progressors	219	76	65	83	87	141	111
	66–2336	17–626	15–371	14–493	22–3708	46–4206	17–1667
	—	0·002	0·003	0·002	0·04	NS	NS
Progressors	599	257	137	122			
	160–3746	38–817	14–460	14–750			
	—	0·03	0·03	0·03			
CD4+ counts ($\times 10^9$/l)							
All subjects	0·35	0·35	0·45	0·3			
	0·2–0·8	0·2–1·1	0·1–0·9	0·1–0·7			
	—	NS	0·02	NS			
Non-progressors	0·4	0·5	0·7	0·4	0·3	0·4	0·3
	0·2–0·8	0·2–1·1	0·2–0·9	0·2–0·7	0·3–0·8	0·2–0·5	0·2–0·6
	—	NS	0·02	NS	NS	NS	NS
Progressors	0·25	0·25	0·35	0·25			
	0·2–0·4	0·2–0·4	0·1–0·5	0·1–0·4			
	—	NS	NS	NS			

β_2-microglobulin assay Serum β_2-microglobulin was measured by a commercially available quantitative competitive enzyme immunoassay (Pharmacia Diagnostics AB, Uppsala, Sweden).

HIV-1 p24 antigen detection Serum samples were assayed by a commercially available solid-phase sandwich-type enzyme immunoassay (Abbott Laboratories, North Chicago, Ill, USA).

Detection of CD4+ lymphocytes CD4+ lymphocytes were enumerated by an indirect or direct immunofluorescence technique using monoclonal antibodies and a flow cytometry system, as previously described.[16]

Statistical analysis Statistical significance of changes in β_2-microglobulin concentration, p24 antigen concentration and CD4+ lymphocyte count was assessed by the Wilcoxon signed rank test.[17] To assess differences between subgroups of subjects the Wilcoxon rank sum test was used.[17] To investigate whether there was a correlation between the individual changes in β_2-microglobulin concentrations, p24 antigen concentrations and CD4+ cell counts we used Spearman rank correlation coefficients.[17]

Results

All subjects

Serum β_2-microglobulin levels, serum p24 antigen levels and CD4+ cell counts (medians, ranges and p values compared with pretreatment values) of the 18 subjects are shown in table 1.

Median serum β_2-microglobulin levels were 2·5 mg/l at entry, 2·3 mg/l at week 12, 2·4 mg/l at week 24 and 2·4 mg/l at week 48. A decrease was found at week 12 compared with week 0 ($p = 0{\cdot}001$). No obvious benefit was obtained with the addition of acyclovir to zidovudine treatment, neither on clinical outcome (2/6 subjects treated with this combination progressed to AIDS), nor on laboratory results (β_2-microglobulin levels, p24 antigen levels or CD4+ cell counts).

Non-progressors

Serum β_2-microglobulin levels, serum p24 antigen levels and CD4+ cell counts of the 12 subjects who remained asymptomatic during the follow-up period, are depicted in table 1 and in fig 1A, B and C.

At week 0 the median serum β_2-microglobulin level in this group was 2·3 mg/l, it declined to 2·1 mg/l at week 12 and it was 2·6 mg/l at week 120. A decrease was seen at week 12 ($p = 0{\cdot}01$), and an increase at week 96 ($p = 0{\cdot}01$) compared with week 0.

Disease progressors

For the six subjects who progressed to AIDS analysis was performed from week 0 until week 48 and separately at the three last evaluation points before the diagnosis of AIDS was made. Serum β_2-microglobulin levels, serum p24 antigen levels and CD4+ cell counts of these subjects for the first 48 weeks of therapy are depicted in table 1 and fig 2A, B and C; for the last 3 evaluation points before the

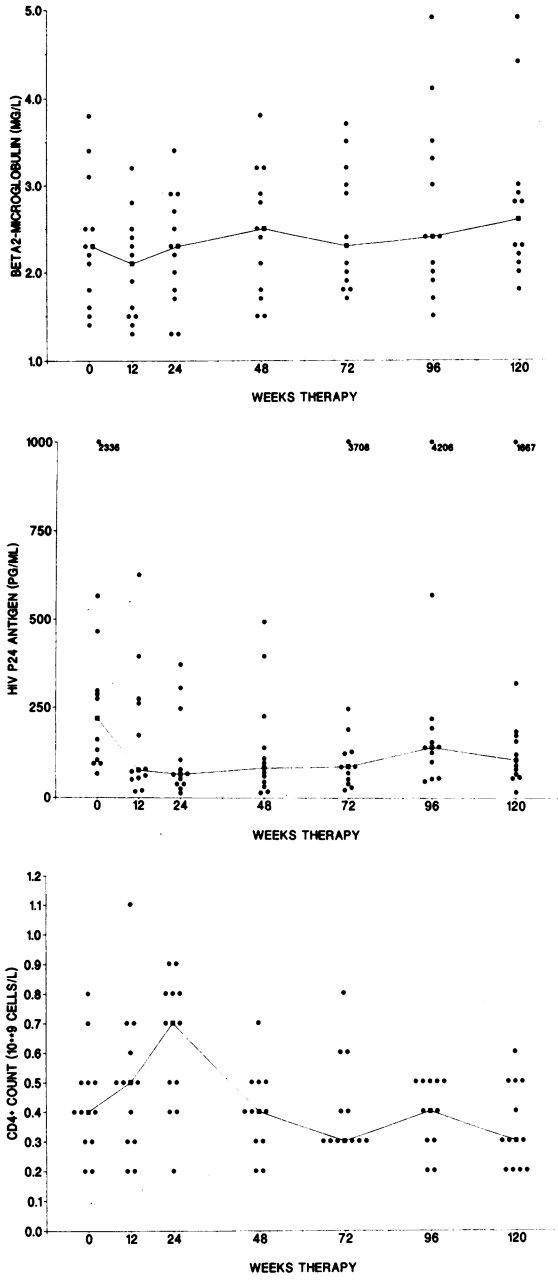

Figure 1 A, B and C. Serum β_2-microglobulin levels, serum p24 antigen levels and CD4+ cell counts in the non-progressors from week 0 until week 120 (■ medians).

Table 2 Serum β_2-microglobulin levels, serum p24 antigen levels and CD4+ cell counts in the disease progressors (n = 6) at an average of 61, 37 and 13 weeks before the diagnosis of AIDS; medians, ranges and p values compared with week −13 (Wilcoxon signed rank test)

Week	*−61*	*−37*	*−13*
β_2-microglobulin (mg/l)			
	2·5	3·0	3·3
	1·9–3·0	1·7–3·9	2·6–4·5
	0·03	0·03	—
P24 antigen (pg/ml)			
	131	178	101
	15–669	14–750	0–663
	NS	0·05	—
CD4+ counts ($\times 10^9$/l)			
	0·3	0·2	0·08
	0·2–0·5	0·1–0·3	0·03–0·1
	0·03	0·04	—

diagnosis of AIDS they are depicted in table 2 and in fig 2A, B and C.

The median serum β_2-microglobulin level decreased from 2·8 mg/l at entry to 2·4 mg/l at week 12 (p = 0·05). These levels were 2·5 mg/l at week 24 and 2·4 mg/l at week 48. Sixty one weeks before AIDS was diagnosed (week −61) it was 2·5 mg/l, increasing to 3·0 mg/l at week −37 and further to 3·3 mg/l at week −13. At week −13 β_2-microglobulin levels were higher than at weeks −37 and −61 (p = 0·03 and p = 0·03).

Comparison between non-progressors and disease progressors

The median serum β_2-microglobulin levels at week 0, 12 and 24 were higher in the group of progressors than in the group of non-progressors, at week 48 it was the other way around; these differences were not statistically significant.

Although median serum p24 antigen levels at weeks 0, 12, 24 and 48 were higher in the group of progressors than in the group of non-progressors, no statistically significant differences could be shown.

CD4+ cell counts at week 0, 12, 24 and 48 were lower in the group of progressors than in the group of non-progressors (p values respectively 0·04, 0·03, 0·01 and 0·05).

Correlations between individual changes in markers

A correlation was found between individual changes in β_2-microglobulin levels and individual changes in p24 antigen concentrations of the 18 subjects at weeks 12, 24 and 48 compared to week 0. The correlation coefficients were $\rho = 0{\cdot}48$, $\rho = 0{\cdot}59$ and $\rho = 0{\cdot}52$ ($p < 0{\cdot}05$). In the same period such a correlation was not found between individual changes in CD4+ cell counts and individual changes in β_2-microglobulin levels or p24 antigen concentrations of the 18 subjects; also such a correlation was not found when the groups of non-progressors and progressors were analysed separately. These two last analyses were performed because of the differences in CD4+ cell counts between both groups.

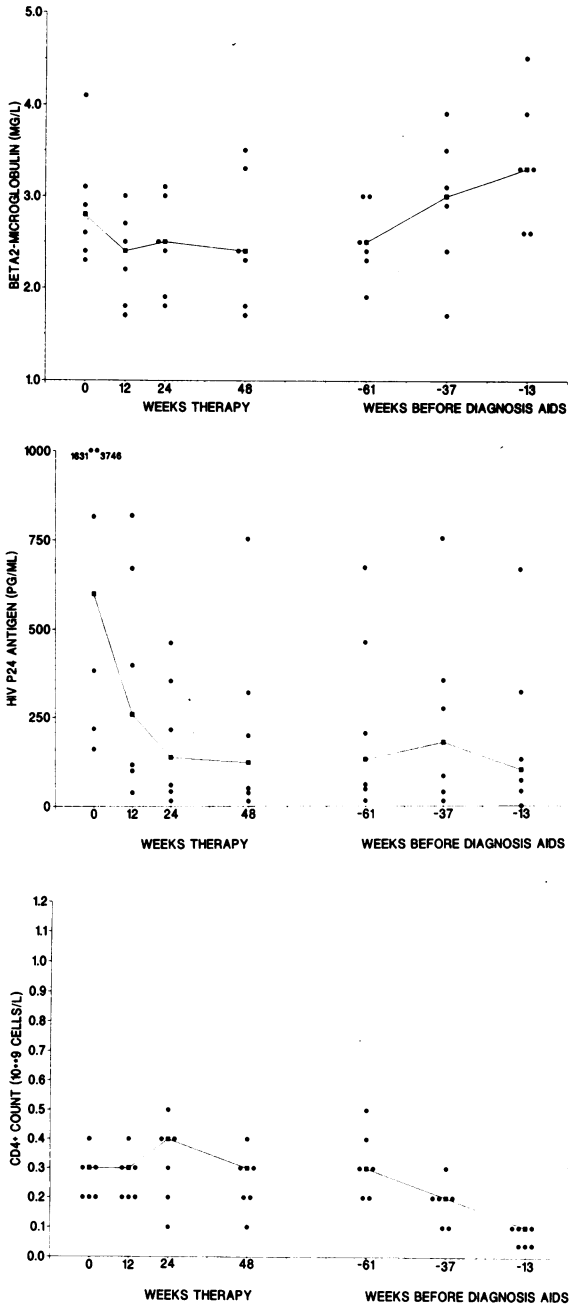

Figure 2 A, B and C. Serum β_2-microglobulin levels, serum p24 antigen levels and CD4+ cell counts in the disease progressors from week 0 until week 48 and at an average of 61, 37 and 13 weeks before the diagnosis of AIDS (■ medians).

Discussion

A statistically significant decline in serum

β_2-microglobulin levels was seen after 12 weeks of treatment in the 18 initially asymptomatic HIV-1 p24 antigen seropositive subjects. The same has been found in zidovudine-treated asymptomatic HIV-1-infected p24 antigen seronegative subjects (J M A Lange, personal communication). In our study also a correlation was found between individual changes in serum β_2-microglobulin levels and individual changes in serum p24 antigen levels during the first 48 weeks of treatment. Those results are consistent with the findings in zidovudine-treated AIDS and ARC patients.[13] It thus appears that β_2-microglobulin can be used as a surrogate marker for monitoring zidovudine therapy in groups of asymptomatic HIV-1-infected subjects. However, one has to be prudent in using β_2-microglobulin as a sole marker for monitoring treatment. In a group of patients with Kaposi's sarcoma successfully treated with alpha-interferon a statistically significant decrease in serum p24 antigen levels was seen, while serum β_2-microglobulin levels initially showed an increase.[18] Moreover, in the present study we did not find a correlation between individual changes in CD4+ cell counts and β_2-microglobulin levels or p24 antigen concentrations.

In the group of the 12 non-progressors the median serum β_2-microglobulin levels and the median CD4+ cell counts from 48 weeks until 120 weeks of treatment showed no statistically significant changes, while in the group of six progressors from 61 weeks until 13 weeks before the diagnosis of AIDS a rise in serum β_2-microglobulin levels and a decline in CD4+ cell counts were found. Serum p24 antigen levels showed an inconsistent pattern after prolonged treatment, both in the subjects who remained asymptomatic and in those who progressed to AIDS. An increase in p24 antigen levels is possibly related to the emergence of HIV-1 strains with reduced sensitivity to zidovudine; after 2 years of treatment a mutation at residue 215 of reverse transcriptase, which is associated with reduced drug sensitivity[19] was found in 16 of the 18 subjects in this group.[20] A decrease in p24 antigen levels however does not rule out drug resistance[20] and may be a consequence of severe CD4+ cell depletion.

In individual subjects the predictive value of the absolute level of serum β_2-microglobulin for disease progression seems to be limited. Three out of the 12 non-progressors had serum β_2-microglobulin levels $\geq$ 3 mg/l at entry and this pattern of distribution was the same after 48 weeks and 120 weeks of treatment. Four disease progressors had levels below 3 mg/l at entry and 2 of them still had values below 3 mg/l at the last evaluation point before the diagnosis of AIDS.

In conclusion, the variability in serum β_2-microglobulin levels appears to make this marker unsuitable for management decisions in individuals, but β_2-microglobulin can be useful as a surrogate marker for monitoring zidovudine treatment in groups of asymptomatic HIV-1-infected subjects. Measuring serum β_2-microglobulin is simple, inexpensive and possible in every subject, while serum p24 antigen is detectable only in a minority of asymptomatic HIV-1-infected subjects.[4 6-8] However, in increase in β_2-microglobulin levels does not always mean a lack of inhibition of HIV-1 replication, as has been shown in patients treated with alpha-interferon.[18] In analysing the short-term effects of potential antiretroviral drugs in phase 1 and 2 trials the combined measuring of serum β_2-microglobulin levels, CD4+ cell counts and, if detectable, serum p24 antigen levels can be used. For monitoring the long-term effects of zidovudine in our phase 2 study of asymptomatic HIV-1-infected subjects, serum p24 antigen levels proved not to be useful, but after prolonged treatment both serum β_2-microglobulin levels and CD4+ cell counts appeared to have predictive value for disease progression.

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